AMENDMENTS

In the specification:

Please amend the paragraph beginning on page 5, containing lines 33-35 and ending on page 6, containing lines 1-13, as follows:

(Amended)

Transactivation of other viral early genes in permissive infection of human cells is principally mediated by the amino acid sequence encoded in the CR3 region of E1A (Lillie et al., 1986 Cell 46:1043-1051). Conserved cysteine residues in a $CysX_2CysX_{13}CysX_2Cys$ sequence motif (SEQ ID NO: 30) in the unique region are associated with metal ion binding activity (Berg, 1986 supra) and are essential for transactivation activity (Jelsma et al., 1988 Virology 163:494-502; Culp et el., 1988 PNAS, USA 85:6450-6454). As well, the amino acids in CR3 which are immediately amino (N)-terminal to the metal binding domain have been shown to be important in transcription activation, while those immediately carboxy (C)-terminal to the metal binding domain are important in forming associations with the promoter region (Lillie and Green, 1989 Nature 338:39-44; see Fig. 3).

Please amend the paragraph on page 11, containing lines 20-34, as follows:

(Amended)

Brief Descriptions of the Drawings

Figure 1A-1L. Sequence and major open reading frames of the left 11% of the BAV3 genome (SEQ ID NO: 1 through SEQ ID NO: 8). The region comprises the E1 and protein IX transcription region. The 195 nucleotide inverted terminal repeat sequence identified by Shinagawa et al., 1987 Gene 55:85-93 is shown in *italics*. The amino acid sequence for the largest E1A protein, two E1B proteins and IX are presented. The probable splice donor ([), splice acceptor (]) and intron sequence (*underlined italics*) within the E1A region are marked. A 35 base pair repeat sequence between E1A and E1B is indicated in **bold underline**. Possible transcription promoter **TATA** sequences and possible poly A addition sequences **AATAA** are also indicated.

Please amend the paragraph on page 12, containing lines 1-12, as follows:

(Amended)

Figure 2A-2B. Regions of homology in the E1A porteins of BAV3 and human adenovirus type 5 (HAd5). The amino acid residue of each serotype is indicated. A. Conserved region 3 (CR3) of HAd5 (SEQ ID NO: 9) subdivided into three functional regions as defined by Lillie et al (1989) Nature 338:39-44 and described in the Backround of the Invention. The intron sequence of BAV3 E1A occurs within the serine amino acid codon at position 204 (nucleotide positions 1216-1322 of SEQ ID NO: 1). B. A portion of conserved region 2 (CR2) of HAd5(SEQ ID NO: 10), showing the residues thought to be important in the binding of retinoblastoma protein Rb (Dyson et al., 1990 J. Virol. 64:1353-1356), and the comparable sequence from BAV3(SEQ ID NO: 34).

Please amend the paragraph on page 12, containing lines 13-16, as follows:

(Amended)

Figure 3A-3B. Homology regions between the HAd5 and E1B 19k (176R) protein (SEQ ID NO: 11 and SEQ ID NO: 12) and the corresponding BAV3 (157R) protein (amino acid positions 83-99 and 136-142 of SEQ ID NO: 4). The amino Acid residue number for each of the viruses is indicated.

Please amend the paragraph on page 12, containing lines 17-22, as follows:

(Amended)

Figure 4A-4B. The C-terminal 346R of HAd5 E1B 56k (496R) (SEQ ID NO: 13) and the corresponding BAV3 protein (420R) (amino acid positions 74-420 of SEQ ID NO: 6). The HAd5 protein comparison begins at residue 150 and the BAV3 (in italics) at residue 74. The amino terminal regions of these proteins which are not presented show no significant homology.

Please amend the paragraph on page 12, containing lines 23-25, as follows:

(Amended)

Figure 5. Homology comparison of the amino acid sequence of HAd5 protein IX (SEQ ID NO: 14) and the corresponding protein of BAV3 (SEQ ID NO: 8) (in italics).

Please amend the paragraph on page 13, containing lines 1-8, as follows:

(Amended)

Figure 7A-7R. Nucleotide sequence of BAV3 between 77 and 92m.u. (SEQ ID NO: 15 through SEQ ID NO: 26) showing ORFs that have the potential to encode polypeptides of at least 50 amino acids after the initiating methionine. The nucleotide sequence was analyzed using the program DISPCOD (PC/GENE). Potential N-glycosylation sites (N-X-T/S) and polyadenylation signals are underlined and the first methionine of each ORF is shown in bold.

Please amend the paragraph on page 13, containing lines 9-20, as follows:

(Amended)

Figure 8A-8C-3. Comparison between the predicted amino acid sequences for the ORFs of BAV3 and known proteins of HAd2 or -5 using the computer program PALIGEN (PC/GENE), with comparison matrix structural-genetic matrix; open gap cost 6; unit gap cost 2. Identical residues are indicated by a colon and similar residues by a dot. (a) Comparison between the predicted amino acid sequence encoded by the 3' end of BAV3 ORF 1 (SEQ ID NO: 16) and the HAd2 hexon-associated pVIII precursor (SEQ ID NO: 27). (b) Comparison between the ORF 4 (amino acid positions 34-154 of SEQ ID NO: 22) and the HAd5 14.7K E3 (SEQ ID NO: 28) protein. (c) Comparison between the predicted amino acid sequence encoded by BAV3 ORF 6 (amino acid positions 8-983 of SEQ ID NO: 26) and the HAd2 fibre protein (SEQ ID NO: 29).

The paragraph at page 15, containing lines 13-34 has been amended, as follows:

(Amended)

Figure 11A-11B. Southern blot analyses of restriction enzymes digested DNA fragments of the wt BAV3 or recombinant genomes by using a 696 bp XhoI-NcoI fragment from pSM14 (Fig. 9) and a DNA fragment containing the luciferase gene as probes. 100 ng DNA isolated from the mock (lanes 1, 2, 3), BAV3-Luc (3.1) (lanes 4, 5, 6), BAV3-Luc (3.2) (lanes 7, 8, 9) or wt BAV3 (lanes 10, 11 12)-infected MDBK cells were digested with BamHI (lanes 1, 4, 7, 10), EcoRI (lanes 2, 5, 8, 11) or XbaI (lanes 3, 6, 9, 12) and analyzed by agarose gel electrophoresis. The DNA fragments from the gel were transferred onto a *GeneScreenPlus*™ membrane and hybridized with a 696 bp XhoI-NcoI fragment from pSM14 (Fig. 9) labeled with ^{32}P using

Pharmacia Oligolabeling Kit (panel A). Panel B blot represents duplicate samples as in panel A but was probed with a 1716 bp BsmI-SspI fragment containing the luciferase gene (Fig. 9). The sizes of bands visualized following hybridization are shown in kb on the right in panel A and on the left in panel B.

B: BamHI, E: EcoRI, Xb: XbaI, 3.1: BAV3-Luc (3.1), 3.2: BAV3-Luc (3.2) and wt: wild-type BAV3.

The paragraph at page 16, containing lines 21-30 has been amended, as follows:

(Amended)

Figure 14A-14B. Luciferase expression in the presence of 1-β-D-arabinofluranosyl cytosine (AraC) in MDBK cells-infected with BAV3-Luc. Confluent MDBK cell monolayers in 25 mm multi-well culture plates were infected with A) BAV3-Luc (3.1) or B) BAV3-Luc (3.2) at a m.o.i. of 50 p.f.u. per cell and incubated in the absence or presence of 50 μg AraC per ml of maintenance medium. At indicated time points post-infection, virus-infected cells were harvested and assayed in duplicate for luciferase activity.

The paragraph beginning at page 16, containing lines 31-35 and ending on page 17, containing lines 1-14 has been amended, as follows:

(Amended)

Figure 15A-15B. Transcription maps of the wt BAV3 and BAV3-Luc genomes in the E3 region. The genome of wt BAV3 between m.u. 77 and 82 is shown which represents the E3 region. The location of XhoI and NcoI sites which were used to make an E3 deletion are shown. (a) The three frames (F1, F2 and F3) representing the open reading frames (ORFs) in the upper strand of the wt BAV3 genome in the E3 region are represented by bars. The shaded portions indicate regions of similarities to pVIII and E3-14.7 kDa proteins of HAd5. The positions of the initiation and termination codons for ORFs likely to code for viral proteins are shown by open and closed triangles, respectively. (b) The predicted ORFs for the upper strand in E3 of the BAV3-Luc genome are shown after a 696 bp XhoI-NcoI E3 deletion replaced by the luciferase gene. The ORFs for pVIII and E3-14.7 kDa proteins are intact. The transcription map of the wt BAV3 E3 was adapted from the DNA sequence submitted to the GenBank database under accession number D16839.

Please amend the paragraph beginning on page 38, containing lines 1-35 and ending on page 39, containing lines 1-14, as follows:

(Amended)

Example 2 Coding Sequences of the BAV3 El-Region

BAV3 genomic DNA, from the left end of the genome to the HindIII site at approximately 11%, was cloned into plasmids and sequenced by a combination of manual and automated sequencing. An examination of the resultant BAV3 E1 genomic sequence (Fig 1) revealed a number of interesting features relevant both to transactivation and to other functions associated with adenovirus El proteins. On the basis of open reading frames (ORFs) it was possible to assign potential coding regions analogous to those defined in human Ad5 (HAd5). As shown in Fig 1, ORFs corresponding roughly to the first exon and unique region of HAd5 ElA as well are ORFs corresponding to the 19k and 58k proteins of E1B and the ORF corresponding to protein IX were all defined in this sequence. The open reading frame defining the probable E1A coding region begins at the ATG at nt 606 and continues to a probable splice donor site at position 1215. The first consensus splice acceptor site after this is located after nt 1322 and defines an intron of 107 base pairs with an internal consensus splice branching site at position 1292. The putative BAV3 E1A polypeptide encoded by a message corresponding to these splice sites would have 211 amino acids and a unmodified molecular weight of 23,323. The major homology of the protein encoded by this ORF and HAd5 ElA is in the residues corresponding to CR3 (shown in Fig 2). The homology of amino acid sequences on both sides of the putative intron strengthens the assignment of probable splice donor and acceptor sites. The CR3 has been shown to be of prime importance in the transactivation activity of HAd5 EIA gene products. As seen in Fig. 2A the homology of this sequence in the BAV3 protein to the corresponding region of the 289R ElA protein of HAd5 includes complete conservation of the $CysX_2CysX_{13}CysX_2Cys$ sequence motif (SEQ ID NO: 30) which defines the metal binding site of this protein (Berg, 1986 Science 232:485-487) as well as conservation of a number of amino acids within this region and within the promoter binding region as defined by Lillie and Green 1989 Nature 338:39-44).

Please amend the paragraph beginning on page 41, containing lines 28-35 and ending on page 43, containing lines 1-8, as follows:

(Amended)

In keeping with the general organization of the E1A region of other adenoviruses, the BAV3 E1A region contains an intron sequence with translation termination codons in all three reading frames and which is therefore probably deleted by splicing from all E1A mRNA transcripts. The largest possible protein produced from the BAV3 E1A region will have 211 amino acid residues and is the equivalent of the 289 amino acid protein translated from the 13s mRNA of HAd5. Two striking features in a comparison of these proteins are the high degree of homology in a region corresponding to CR3 and the absence in BAV3 of most of amino acids corresponding to the second exon of HAd5. In fact the only amino acids encoded in the second exon of BAV3 are, those which are considered to constitute part of CR3. A great deal of work carried out with HAd5 has identified the importance of the CR3 sequences in transactivation of other HAd5 genes. While a detailed analysis of the corresponding BAV3 region and its possible role in transactivation of BAV3 genes needs to be carried out, it is none-the-less interesting to note a couple of possibly pertinent features. The HAd5 CR3 region has been operationally subdivided into three regions (Lillie et al, 1989 Nature 338:39-44; see Fig 8); an N-terminal region from 139 to 153 which has four acidic residues and is thought to be important in transcription activation, a central, metal-binding, region defined by the Cys-X_2-Cys-X_{13}-CysX_2-Cys sequence (SEQ ID NO: 30) which is essential for both promoter binding and activation, and a C-terminal region (residues 175-189) which is essential for promoter binding. Since, in most instances, E1A protein is thought not to interact directly with DNA (Ferguson et al 1985), the promoter binding regions may be involved in forming associations with proteins which then allow association with DNA. In Fig 2a the BAV3 E1A protein contains the central, metal binding domain and has considerable homology in the carboxy portion of this region. The BAV3 E1A protein also shows identity of sequence with HAd5 in the carboxy 6 amino acids of the promoter binding domain. These features may allow the BAV3 E1A protein to interact with the same transcription activating factors required for HAd5 E1A function. In contrast, except for a Glu-Glu pair there is little homology between the bovine and human viruses in the activation domain. The fact that this domain can be functionally substituted by a heterologous acidic activation sequence (Lillie et al, 1989 supra) suggests that protein specificity is not required in this region and this

may allow the BAV3 E1A protein to function in the activation of BAV3 genes. The BAV3 E1A activation region contains six acidic residues in the 18 residues amino to the metal binding domain.

Please amend the paragraph on page 44, containing lines 1-26, as follows:

(Amended)

HAd5 E1B encodes two proteins (19k and 56k) either of which can cooperate with E1A, by pathways which are additive and therefore presumably independent (McLorie et al, 1991 J. Gen. Virol. 72:1467-1471), to produce morphological transformation of cells in culture (see for example: Branton et al, 1985 supra; Graham, 1984 supra). The significance of the conservation of the hydrophobic stretch of amino acids in the central portion of the shorter E1B proteins of HAd5 and BAV3 is not clear as yet. A second short region of homology Gln-Ser-Ser-X-Ser-Thr-Ser (SEQ ID NO: 31) at residue 136 near the C-terminus of the BAV3 protein is located near the N-terminus at residue 20 in the HAd5 19k protein. The major difference in both length and sequence of the larger (420R) E1B protein of BAV3 from the corresponding HAd5 protein (496R) is confined to the N-terminus of these proteins. The two proteins show considerable evolutionary homology in the 345 amino acids that extend to their C-termini. A similar degree of homology extends into the N-terminal halves of protein IX of BAV3 and HAd5. Taken together these analyses suggest that while BAV3 and the human adenoviruses have diverged by simple point mutational events in some regions, more dramatic genetic events such as deletion and recombination may have been operating in other regions particularly those defining the junction between E1A and E1B.

Please amend the paragraph beginning on page 47, containing lines 28-35 and ending on page 48, containing lines 1-34, as follows:

(Amended)

HAd2 and HAd5 E3 lies between the pVIII and the fibre genes an encodes at least 10 polypeptides (Cladaras & Wold, 1985, supra). The promoter for E3 of these two serotypes lies within the sequences encoding pVIII, about 320 bp 5' of the termination codon. No consensus TATA box is found in the corresponding region of the BAV3 sequences. A non-canonical polyadenylation signal (ATAAA) for E3 transcripts is located at position 1723, between the end